Gene Therapy for Pediatric Diseases

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Assignment: The sequencing of the human genome has led to hope and expectation of gene therapy for genetic disorders. Reports of success in treating hemophilia, Leber congenital amaurosis, leukemia, and severe combined immunodeficiency will lead to the expectation that this will soon be available for all genetic disorders.

This session will provide the pediatrician with a solid understanding of the success, and limitations, of gene therapy thus far and which types of disorders will be most likely to be treated in this novel manner in the near future.

Disclosure Statement

Actelion – Speakers Bureau
Amicus Therapeutics – Meeting Sponsor Relationship
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BioMarin – Research Grant
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Prisacta – Speakers Bureau
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• Gene Therapy for Metabolic Disorders
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Synageva – Meeting Sponsor
University of Minnesota
• Department of Pediatrics
• Center for Translational Science Institute (CTSI)
Zebra c.b.a. A WORLD Symposium (Feb 3-5, 2015, Orlando, FL, USA)
“We’re Organizing Research on Lysosomal Diseases”

This presentation may discuss off-label use of FDA-approved medications.

Rare Diseases Clinical Research Network (RDCRN)

Lysosomal Diseases

• Aspartylglucosaminuria
• Wolman disease
• Cystinosis
• Danon disease
• Fabry disease
• Farber disease
• Fucosidosis
• Gaucher disease
• GM1-Gangliosidosis types I and II
• GM2-Gangliosidosis
• α-Mannosidosis types I and II
• Mannosidosis
• Metachromatic leukodystrophy
• Niemann-Pick disease
• Pseudo-Hurler polydystrophy
• Schindler disease
• Sly syndrome
• Tay-Sachs disease
• Type A Fabry disease
• Type B Fabry disease
• Type C Fabry disease
• Type III Fabry disease

• Hunter syndrome
• Sanfilippo syndrome types A, B, C and D
• Gaucher disease infantile
• Krabbe disease
• Niemann-Pick disease
• Farber disease
• Pseudo-Hurler polydystrophy
• Sandhoff disease
• Morquio syndrome
• Maroteaux-Lamy syndrome
• Morquio-Behr syndrome
• Morquio-A syndrome
• Morquio-B syndrome
• Morquio-C syndrome
• Morquio-D syndrome
• Morquio-H syndrome
• Morquio-V syndrome
• Morquio-W syndrome
• Morquio-X syndrome
• Morquio-Y syndrome
• Multiple sulfatase deficiency
• Batten disease
• Tay-Sachs disease
• Pompe disease
• Batten diseases
• Late infantile Northern episcleritis
• Pycnodysostosis
• Schindler disease
• Salla disease
• Saint-Aubert disease
• Salla disease
Milestones in Gene Therapy 1972 “Gene therapy” coined by Ted Friedmann

1973 Self-imposed moratorium on recombinant DNA research by the molecular biology scientific community

1975 Asilomar Conference on Recombinant DNA leading to establishment of the NIH Recombinant DNA Committee (RAC)

Gene Therapy (AAVrh.10-SGSH)

Genetic Engineering and Gene Therapy c. 1972

“...gene therapy may ameliorate some human genetic diseases in the future. For this reason, we believe that research directed at the development of techniques for gene therapy should continue. For the foreseeable future, however, we oppose any further attempts at gene therapy in human patients because:

(i) Our understanding of such basic processes as gene regulation and genetic recombination in human cells is inadequate;

(ii) Our understanding of the details of the relation between the molecular defect and the disease state is rudimentary for essentially all genetic diseases; and

(iii) We have no information on the short-range and long-term side effects of gene therapy.


We therefore propose that a sustained effort be made to formulate a complete set of ethico-scientific criteria to guide the development and clinical application of gene therapy techniques. Such an endeavor could go a long way toward ensuring that gene therapy is used in humans only in those instances where it will prove beneficial, and toward preventing its misuse through premature application.

NIH Recombinant DNA Advisory Committee (RAC) Human Gene Therapy Protocols
9007-002
Blaese, R. Michael; National Institutes of Health, Bethesda, Maryland; Treatment of Severe Combined Immunodeficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with Autologous Lymphocytes Transduced with the Human ADA Gene: An Experimental Study
*RAC Recommends Approval: 7-31-90/NIH Approval: 9-6-90
Gene Therapy/Phase I/Monogenic Disease/Severe Combined Immunodeficiency due to Adenosine Deaminase Deficiency/In Vitro/Autologous Peripheral Blood Cells/Cord Blood/Placenta Cells/Retrovirus/Adenosine Deaminase cDNA/Neomycin Phosphotransferase cDNA/Intravenous

NIH Recombinant DNA Advisory Committee (RAC) Human Gene Therapy Protocols
9012-139
Batshaw, Mark; Institute for Human Gene Therapy, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania; A Phase I Study of Adenoviral Vector Mediated Gene Transfer to Liver in Adults with Partial Ornithine Transcarbamylase Deficiency
Gene Therapy/Phase I/Monogenic Disease/Partial Ornithine Transcarbamylase (OTC) Deficiency/In Vivo/Autologous Peripheral Blood Cells/Adenovirus/Type 5 (E2a Temperature-Sensitive Mutant)/Ornithine Transcarbamylase (OTC) Deficiency/Partial Ornithine Transcarbamylase (OTC) Deficiency/In Vivo/Autologous Peripheral Blood Cells/Adenovirus/Type 5 (E2a Temperature-Sensitive Mutant)
cDNA/Intravenous

NIH Recombinant DNA Advisory Committee (RAC) Human Gene Therapy Protocols
9512-139
Batshaw, Mark; Institute for Human Gene Therapy, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania; A Phase I Study of Adenoviral Vector Mediated Gene Transfer to Liver in Adults with Partial Ornithine Transcarbamylase Deficiency
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cDNA/Intravenous

NIH Recombinant DNA Advisory Committee (RAC) Human Gene Therapy Protocols
0312-199
Chester, Ronald, Weill Medical College of Cornell University, New York, New York; Administration of a Replication Deficient Adeno-Associated Virus Gene Transfer Vector Expressing the Human CLN2 cDNA to the Brain of Children with Late Infantile Neuronal Ceroid Lipofuscinosis
Gene Therapy/Monogenic Disease/Late Infantile Neuronal Ceroid Lipofuscinosis/In Vivo/Brain/Adeno-Associated Virus 2/CLN2 (Tripeptidyl Peptidase)
cDNA/Intraparenchymal Injection
Emerging Trends in Gene Therapy

- Glybera approval by European Commission
- Leber congenital amaurosis
- Lentiviral-mediated hematopoietic cell transplantation (ADA-deficient SCID, Don Kohn)
- Intravenous lentiviral gene therapy for Hurler syndrome
- Sanfilippo syndrome type A, AAV injection into the brain, Lysogene
- Giant axonal neuropathy, AAV intrathecal injection

Leber Congenital Amaurosis

The first retinal gene therapy in human blindness from RPE65 mutations has focused on safety and efficacy, as defined by improved vision. The disease component not studied, however, has been the fate of photoreceptors in this progressive retinal degeneration. We show that gene therapy improves vision for at least 3 y, but photoreceptor degeneration progresses unabated in humans... The study shows the need for combinatorial therapy to improve vision in the short term but also slow retinal degeneration in the long term.
Gene Therapy Using Hematopoietic Stem Cells

First and Second Generation Retroviral and Lentiviral Vectors

Phase I/II Trial: EFS-ADA Lentiviral Vector

<table>
<thead>
<tr>
<th>ID</th>
<th>Age at entry</th>
<th>Gender</th>
<th>Race</th>
<th>CD34+ x 10^6/kg</th>
<th>VCN</th>
<th>Busulfan AUC (mmol/L*min)</th>
<th>Months on Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>501U</td>
<td>7 mos</td>
<td>F</td>
<td>H</td>
<td>11.1</td>
<td>2.9</td>
<td>5,673</td>
<td>11 mos</td>
</tr>
<tr>
<td>502U</td>
<td>3.6 y</td>
<td>F</td>
<td>AA</td>
<td>5.6</td>
<td>2.4</td>
<td>4,518</td>
<td>9 mos</td>
</tr>
<tr>
<td>503U</td>
<td>3.0 y</td>
<td>M</td>
<td>H</td>
<td>n.d. (&lt;1)</td>
<td>1.7</td>
<td>n.d.</td>
<td>N/A</td>
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<tr>
<td>504U</td>
<td>4 mos</td>
<td>M</td>
<td>C</td>
<td>15.0</td>
<td>1.6</td>
<td>5,199</td>
<td>4 mos</td>
</tr>
<tr>
<td>505U</td>
<td>9 mos</td>
<td>M</td>
<td>AA</td>
<td>9.0</td>
<td>6.3</td>
<td>3,673</td>
<td>3 mos</td>
</tr>
<tr>
<td>506U</td>
<td>2 y</td>
<td>M</td>
<td>H</td>
<td>3.0</td>
<td>2.8</td>
<td>4,055</td>
<td>2 mos</td>
</tr>
<tr>
<td>507U</td>
<td>10 mos</td>
<td>F</td>
<td>C</td>
<td>15.0</td>
<td></td>
<td>3,247</td>
<td>Pend</td>
</tr>
<tr>
<td>508U</td>
<td>6.5 mos</td>
<td>F</td>
<td>C</td>
<td>Pend</td>
<td>Pend</td>
<td>Pend</td>
<td>Pend</td>
</tr>
</tbody>
</table>

Diagnosed by CA NBS
Adagen started @ 1 mo
Age at Transplant: 8 mo

Final Cell Product:
- EFS-ADA Vector copy/mL
- ADA enzyme activity: 6974 (U)

Busulfan dose: 4mg/kg
Busulfan AUC: 5673 umol/L min
Adagen stopped day +30

Hematopoietic Stem Cell Differentiation

The EFS-ADA Lentiviral Vector

Clinical Trial Experimental Time-Line

Phase I/II Trial: EFS-ADA Lentiviral Vector

<table>
<thead>
<tr>
<th>Event</th>
<th>Time</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consent</td>
<td>Weeks -1</td>
<td>Consent</td>
</tr>
<tr>
<td>Screening</td>
<td>Weeks -2</td>
<td>Screening</td>
</tr>
<tr>
<td>Treatment</td>
<td>Weeks 0</td>
<td>Treatment</td>
</tr>
<tr>
<td>Safety</td>
<td>1st - 2nd Year</td>
<td>Safety</td>
</tr>
<tr>
<td>Long-Term Follow-Up</td>
<td>Years 3 - 15</td>
<td>Long-Term Follow-Up</td>
</tr>
</tbody>
</table>

CD34+
Gene Therapy for Hurler Syndrome

- Hematopoietic stem cell transplant stabilizes disease (1982)
- Donor engraftment halts IQ loss of 1.6 IQ points/month
- Complicated by significant morbidity and mortality

Murine MPS I is Indistinguishable in Newborn Pups but Readily Differentiated in Older Mice

MPS I homozygote ≠ Normal

Hypothesis

Would simple, early intravenous administration of a lentiviral vector to a newborn achieve high levels of gene transfer and gene expression, resulting in correction of MPS I?

Self-inactivating Lentiviral Vector CSP1 for Expression of Human α-L-iduronidase

Four Plasmids for Production of a Third-generation Self-inactivating HIV-based Lentiviral Vector
α-L-Iduronidase Activity in Plasma After IV Lentiviral Gene Therapy

α-L-Iduronidase Activity in MPS I Mice 100 Days After IV Lentiviral Gene Therapy

<table>
<thead>
<tr>
<th>Mice</th>
<th>Liver</th>
<th>Spleen</th>
<th>Brain</th>
<th>Gonad</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>15.1</td>
<td>1.0</td>
<td>0.10</td>
<td>0.23</td>
</tr>
<tr>
<td>55</td>
<td>1.12</td>
<td>1.3</td>
<td>0.09</td>
<td>UD</td>
</tr>
<tr>
<td>56</td>
<td>0.85</td>
<td>1.1</td>
<td>0.06</td>
<td>0.29</td>
</tr>
<tr>
<td>61</td>
<td>50.5</td>
<td>2.7</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>62</td>
<td>67.6</td>
<td>2.9</td>
<td>0.26</td>
<td>0.16</td>
</tr>
<tr>
<td>63</td>
<td>44.3</td>
<td>2.2</td>
<td>0.22</td>
<td>0.07</td>
</tr>
<tr>
<td>64</td>
<td>47.3</td>
<td>2.2</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>65</td>
<td>0.20</td>
<td>0.26</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>66</td>
<td>0.82</td>
<td>0.71</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>67</td>
<td>52</td>
<td>2.47</td>
<td>0.09</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Carrier: 4.0 ± 0.42 14.9 ± 2.4 3.7 ± 0.55 15.8 ± 5.7
UD, undetectable, < 0.01 nmol/mg/hr.

Correction of Craniofacial DYSmorphology in MPS I Mice 100 Days After Neonatal Treatment

Correction of GM2-Ganglioside Accumulation in the Hippocampus of Murine Hurler Syndrome

Murine Hurler Syndrome Liver Pathology

Habituation: Locomotor Activity

* p = 0.064 vs. MPS I
* p = 0.014 vs. Carrier
α-Iduronidase Expression in the Brain of Mice iht Hurler Syndrome after Intravenous Administration of Lentiviral Vector

Sanfilippo Syndrome Type A (Mucopolysaccharidosis Type III A)

- Progressive neurodegenerative disease
- Normal at birth
- Peak developmental age of 28 months
- Survival to 15 - 30 years of age

Lysogene MPS IIIA development overview

- AAV vector serotype rh10
  - Transduces a large area of the CNS with high level of transgene expression (Swain et al. Gene Ther 2013)
  - Favorable immunogenicity profile (Chirmule 1999, Halbert 2006)
- Carrying a normal copy of the N-sulfoglycosamin sulfohydrolase (SGSH) gene
- Intracerebral administered gene therapy using frameless stereotactic guidance: the most advancing and proven strategy in humans (Batten, Canavan trials1, Metachromatic Leukodystrophy2 and Sanfilippo B)3
- Orphan drug status in EU and US

Concept: Correction of defect should reduce/reverse toxic storage, improve functions and health status

Phase I/II Study Design

- Primary objective: to assess tolerance and safety of SAF-301
- Secondary: Definition of clinical endpoints for future efficacy studies (brain imaging, neuropsychological tests and biological markers)
- Open-label non-controlled monocentric study
  - 4 patients treated in a single neurosurgical procedure with one year follow up Aug 2011 – May 2013
  - Immunosuppressive treatment to prevent transduced cells elimination

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>8y 7m</td>
</tr>
<tr>
<td>Patient 2</td>
<td>8y 5m</td>
</tr>
<tr>
<td>Patient 3</td>
<td>8y 10m</td>
</tr>
<tr>
<td>Patient 4</td>
<td>8y 8m</td>
</tr>
</tbody>
</table>

Phase I/II safety results

- Good tolerance
- Neurosurgery itself was uneventful
- Absence of adverse events related to the injected product
- No increase in the number of infectious events
- No biological sign of toxicity related to immunosuppressive drugs

Intracerebral Administration of Adeno-Associated Viral Vector Scorpion/shh Carrying Human GUSB and SULF2 Coding in Children with Mucopolysaccharidosis Type III Disease. Results of a Phase III Trial

1Souweidane et al. J Neurol Ped 2010
2Leone et al. Sci Transl Med 2012
3Clinicaltrials.gov number: NCT01801709
4Sanfilippo Pancrea
**Phase I/II Efficacy Results**

- Neuropsychological evaluations suggested improvement, although moderate, in behaviour and attention in the 3 older patients who showed cognitive decline at inclusion.
- Neurocognitive benefit was more marked in the youngest patient.
- Improvement in behavioural disorders, hyperactivity and sleep, reported by the parents (stopped symptomatic medications).

**Lessons learned**

- Younger patients most likely to benefit from new therapy.
- Improved dosing and administration: cerebellar injections.
- Need quantified validated data on hyperactivity, sleeping disorders and quality of life.

**Giant Axonal Neuropathy (GAN)**

- Rare autosomal recessive disease of the central and peripheral nervous system caused by loss of gigaxonin gene (GAN).
- Loss of gigaxonin protein results in intermediate filament (IF) accumulation.
- Axonal accumulation of IFs causes the most severe disease symptoms.

**Phase II/III Study design**

- Single-arm, non-randomised open-label study including 12 patients worldwide.
- Total dose increased versus SAF-301.
- Additional injection site (cerebellum).
- Anticipate minimum three clinical trial sites in Europe & US.
- Duration: average 2 year follow up post dosing per patient depending on age of patient at inclusion.
- Start: Q4 2015.
- Immunosuppressive treatment.
- European Medicines Agency (EMA) protocole assistance completed 09/14.
- USA: Pre-IND (investigational new drug) procedure.
CONCLUSIONS:
• Intrathecal injection of AAV9 in juvenile pigs resulted in 50–100% of spinal cord motor neuron transduction across the entire spinal cord.

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CONCLUSIONS:
• Intrathecal administration of AAV9 leads to global vector distribution and transgene expression to the CNS.
• Intrathecal administration allows for a lower dose, avoidance of anti-capsid antibodies, and reduced peripheral organ biodistribution.
• This approach translates effectively from rodents to non-human primates.

NIH Recombinant DNA Advisory Committee (RAC)
Human Gene Therapy Protocols
1304-1231
Bonnemann, Carsten; National Institutes of Health; Bethesda, Maryland; A Phase I Study of Intrathecal Administration of scAAV9/Jet-GAN for the Treatment of Giant Axonal Neuropathy. Sponsor: Hannah’s Hope Fund

Giant Axonal Neuropathy/n Vivo/Adenovirus Associated Virus Serotype 9/GAN Gene, Encodes the Protein Gigaxonin/Intrathecal Administration

Summary
Gene Therapy for Pediatric Diseases
• Convergence of the human genome project, and gene therapy technology, have offered hope for the treatment of many genetic disorders for more than 40 years.
• Recent successes in the past few years predict steady, and accelerating progress in the future.
• The successes – and limitations – of gene therapy promise novel treatments particularly for serious, lethal disorders.